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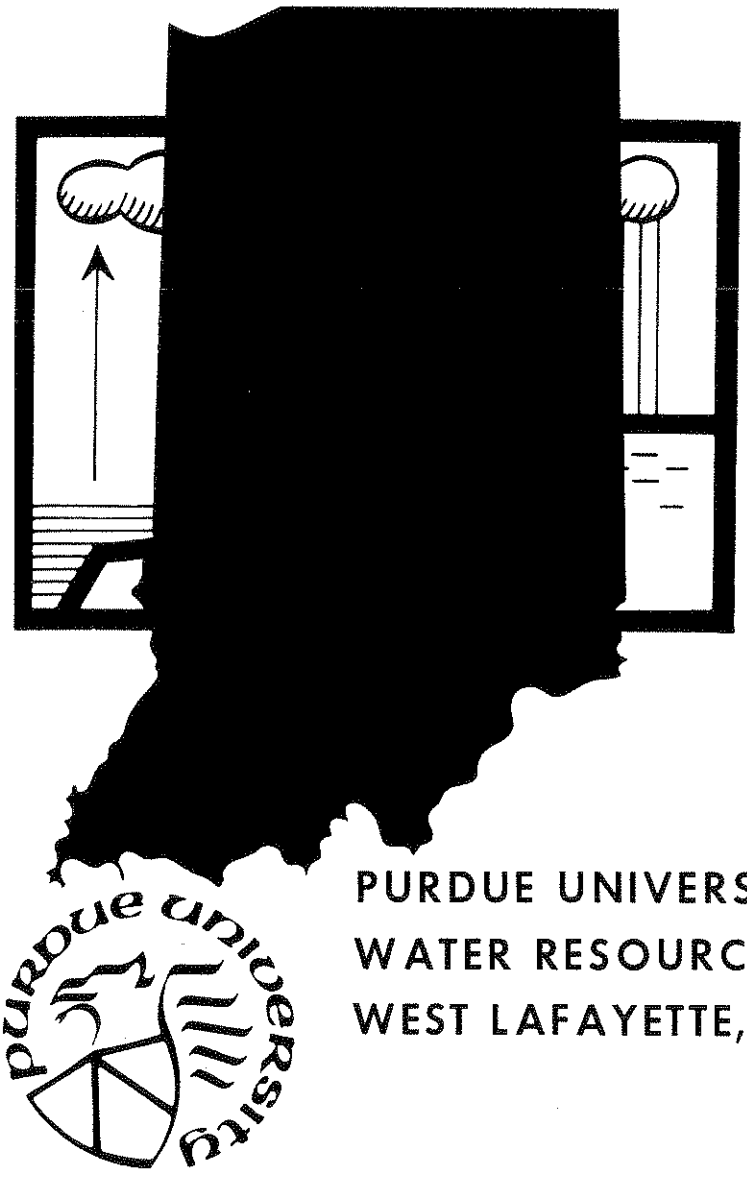
TECHNICAL REPORT NUMBER 185

AGRICULTURAL IMPACT ON GROUNDWATER QUALITY



by

Ronald F. Turco
Allan E. Konopka

June 1988

PURDUE UNIVERSITY
WATER RESOURCES RESEARCH CENTER
WEST LAFAYETTE, INDIANA

Technical Report Number 185

AGRICULTURAL IMPACT ON GROUNDWATER QUALITY

by

R. F. Turco and A. E. Konopka

Project Number: G1421-05

Submitted to:

U.S. Geological Survey
United States Department of the Interior
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Water Resources Research Center
Purdue University, West Lafayette, IN 47907

June 1988

FINAL TECHNICAL COMPLETION REPORT

Period of Investigation: 7/1/87 to 6/30/88

Project Number: G1421-05

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ABSTRACT

Concern over the possible occurrence of pesticides or nitrate in the ground water is justified as nearly 100% of rural Indiana uses ground water for drinking. A study to assess how ground water has been affected by agriculture was initiated. We have monitored 46 wells in 4 counties for pesticides, nitrate and evaluated how placement and depth affects water quality. Our findings indicate that nitrate remains of primary concern. While 30% of the tested wells had elevated levels of N, only 8% would be considered a serious risk. It appears that presence of animals or mishandling of materials in close proximity to the well has the most detrimental effect. With the exception of one site, contamination of well water by agriculture chemicals is not widespread. To better clarify the transformation of pesticides in soil and ground water, we initiated studies to understand how a microorganism is able to utilize aniline, a herbicide intermediate, under single and mixed substrate conditions. Often the pollutant material, such as aniline, is in low concentration as compared to other carbon sources. It appears that our isolated bacteria is capable of simultaneous utilization of aniline and other organic substrates. This is important as it implies that pesticide transformation are possible in the presence of other more utilizable materials.

PART I

INTRODUCTION

The use of both fertilizer and herbicides has increased over the last 20 years. Estimates indicate that in 1986 437,848 mT of N was applied at an average rate of 175 kg ha^{-1} to the fields of Indiana (Gann and Hunst, 1986). In 1987 1.85 x 10^6 ha of corn were treated with herbicide. The percentage of treated hectares includes: 11% Atrazine alone, 17% Atrazine and Dual, 28% Atrazine and Lasso, 3% Atrazine and Bladex, 2% Dual and Lasso alone and 2% Lasso and Bladex. For 1987, 1.70 x 10^6 ha of soybeans were treated with herbicide. The materials applied included among others 6% receiving Lasso, 4% Dual, 3% Treflan, and tank mixes of Treflan, Lasso, and Imazaquin (USDA, 1988). While these materials result in a significant enhancement of crop production, the potential for these materials to cause unwanted environmental affects has only recently come under investigation. One area of great interest is the possibility of the occurrence of these materials in ground and surface water supplies.

Concern for their occurrence in ground water is justified as nearly 100% of rural Indiana uses ground water for a source for drinking water. Further, only a negligible amount of information exist on the level of agricultural contamination in these supplies. The information that does exist is generally spotty and has been collected in response to a known contamination event. This make it difficult to assess the low level or background contamination that may be occurring.

Baker (1980) reviewed a number of the early studies on groundwater contamination and concluded that pesticides were generally either absent, or present at very low levels. However, reported incidents of groundwater

contamination by organics of agricultural origin have increased in recent years (Pye et al., 1983). For the period 1984-1986 Cohen et al. (1986) reported an increase in the number of pesticides found in groundwater from 12 in 18 different states to 17 in 23 states. The most commonly reported xenobiotics (found in > 4 states' aquifers) are Aldicarb, EDB, Atrazine, and DBCP. While concentrations are generally less than 5 ug l^{-1} studies on aldicarb have shown groundwater contamination to be as high as 50 ug l^{-1} (Rajagopal et al., 1984). Other reports from the north central region (Ohio and Iowa) showed atrazine, metolachlor and alachlor occurring at nearly 20 ug l^{-1} (Baker 1985). Reports from Iowa also have indicated that very high levels of both carbofuran and cyanazine are possible. The effects of chronic low level pesticide exposure on human health is unclear (Hoar et al. 1986).

Although the exact mechanism controlling the rate at which pesticides move to groundwater is not clear (Kelly et al., 1986), it may reflect the intermediate binding, transfer through organic matter or movement by macropore flow. This is in contrast to the almost immediate increase in levels of groundwater nitrate following fertilizer applications (Keeney, 1982).

Fertilizer nutrients are a well known source of groundwater contamination. High nitrate concentrations have been found in wells beneath irrigated sands in Georgia (Hubbard, et al., 1984). Studies on nitrate leaching in the north central region indicate a potential for problems as a result of present day fertilizer application methodologies. The inherent inefficiency of plant utilization of applied material (< 50% of applied) contributes to the problem. If we consider that corn receives 150 kg N fertilizer ha^{-1} along with N supplied from soil mineralization, and that the grain removes about 100 kg ha^{-1} the difference must be lost (Haggett and Barry, 1985). Concentrations of N in Ohio field tile water, as an indicator

of downward movement, average 10-30 ug NO_3-N ml^{-1} (Logan et al., 1980). Good quality waters are suggested to be less than 10 ug NO_3-N ml^{-1}. Values as high as 135 mg NO_3-N l^{-1} have been reported in soils under vegetable-cropped fields in California (Lund, 1982).

While fertilizer application has been suggested as a possible source of contamination, it is well documented that feed lots or animal confinement areas do serve as point sources for N. Work by Piskin (1973) showed that feed lots were a significant source of NO_3-N. Others, Terry et al. (1981) have shown the significant impacts that feed lots can have on regions environmental quality. While these studies address the impact of large feed lot operations, few studies to understand the impact of small scale livestock operations have been conducted.

Most well studies are based on single or grab samples; supplemental sampling is conducted only when contamination is found. In general, temporal changes are ignored or factored out. Few efforts have been directed at understanding the impact seasonal changes in contamination. How placement of the well relative to the rest of the farm operation (feedlots, tank washing and herbicide storage) has generally not been considered. An effort to correlate well head activities, contamination and temporal changes should be conducted.

Little is known about levels of contamination in Indiana well water. Even less is known about how well depth and activities near the well, affect the water quality. The research reported in the first phase of this project was undertaken to describe levels of agricultural chemicals in waters sampled taken from three distinct regions of the state.

MATERIALS AND METHODS

Four Indiana counties representing three distinct physiographic regions were chosen for study. These regions included the sandy but poorly drained soils over glacial till or lake deposited sand in the northwest Indiana counties of Newton/Jasper, the poorly drained soils over glacial till in the central region of the state represented by Tippecanoe County and soils of mixed drainage over alluvial deposits or glacial till, found in the southern Jennnings County. These three regions also differ in their potential to serve as ground water supply. Regions in central or northern Indiana can be considered as good to excellent, while sites in Southern Indiana are poor.

Well sites were chosen in a random fashion. However, an attempt was made to cluster or group a number of wells in each county. A total of 10 well clusters, groups of wells within a one mile radius, where established. All well sites were farm supply wells. In order for a well to be included in the study, drilling records indicating depth and age of the well were available.

In addition, owners of the wells were surveyed as to the condition and use of the well. Auxiliary data on water consumption and chemical use was also collected. Included in the survey were questions on the depth and age of the well, the location of the well relative to feed lots or animal handling areas and the type of chemicals commonly used in the operation. Sampling data from farms not returning the survey, was excluded from the study.

A total of 47 wells were sampled beginning in the summer of 1987. Wells were sampled up to 8 different times over the year. Unlike other ag-chemical studies where continued sampling is contingent on the occurrence of contamination, in our study sampling was continual at all sites for the entire time period. While well water was sampled for the entire year, interest in surface waters was developed later in the study resulting in a part year

sampling. Surface water samples were collected beginning in April of 1988. Surface water sampling sites were chosen at random. However, they are in close proximity to our well sites.

Purdue University personnel collected all well and surface water samples. Two well samples were collected at each sampling date. Water for pesticide and nutrient analysis was collected into sulfuric acid-dichromate washed distilled water-acetone rinsed, 1 l clear glass bottles. The caps were also washed and foil lined. For estimations of bacterial numbers, the previously described bottles were used with the addition of an autoclaving step (121°C, 15 atm for 18 mins). A mid-stream sample was collected after allowing the water to flow 5-10 mins. and before it reached any treatment or storage equipment. Once collected, the bottles were capped, placed in a cooler chest packed with ice (4°C) and transported by Purdue personnel to West Lafayette. Surface water samples were collected by submerging one acid washed 1 l clear glass bottle into the flowing stream. Samples were maintained at 4°C and analysis conducted with 48 hours of return to the lab.

Ammonium and nitrate were determined directly on unfiltered water samples using a steam distillation procedure (Keeney and Nelson, 1982). Water, 20 ml, was pipette directly into the apparatus and ammonium-N determined. The reductant magnesium oxide-Devardo alloy was added and nitrate-N determined. Nitrogen was quantified by direct calculation from the amount of acid added to the receiving flask. An Orion pH meter and glass electrode were used to determine pH.

Bacterial counts and coliform type bacteria were determined using a membrane filter method. Water, 10, 50 or 100 ml, was passed through the filter (0.22 um) and the filter transferred to a Mackonkey agar. The filter-agar combination were incubated for 24 hrs at 35°C.

Suspended solids were determined by filtration. Water 750 ml, was passed through a washed, dried and pre-weighted glass-fiber filter (Gelman Type A/E). The filter was removed and dried at 105°C for 1 hour. Suspended solids were determined by difference. The filtered water was used for the remainder of the determinations.

Potassium was determined by direct atomic adsorption (Varian AA-475) measurement. Phosphorus was resolved using an ascorbic acid method (Olson and Summers, 1982). Color development was determined at 880 nm (Bausch and Lomb model 1001). Data was quantified by comparing response against standards of known concentration.

The five pesticides of interest: Trifluralin, Atrazine, Alachlor, Metolachlor, and Cyanazine (Standards from Chem. Service, Pa.) were resolved following extraction onto C18-solid phase column (Sep-Pak, Baker Chm. Co.). This allowed the pesticides to be concentrated from the water samples. The sep-pak columns were activated by pulling, under vacuum, 5 ml of nano-grade methanol over the column. The columns were washed with 10 mls of distilled water, followed without drying by 500 ml of the fiber-filtered well water (flow rate of 2 ml min^{-1}). Columns were allowed to dry (5 hrs) and were eluted with 4 mls of nano-grade benzene. Samples were stored in benzene until analysis. Included with the water samples were periodic sample spikes containing pesticides at known concentrations. Recovery of spikes for the Sep-Pak system was 8.5, 112.6, 104.8, 111.8 and 76.3 for Trifluralin, Atrazine, Alachlor, Metolachlor and Cyanazine, respectively.

At the time of analysis the benzene was removed with N_2 gas and the sample resuspended in 1 ml of nano-grade isooctane. This isooctane was removed with N_2 gas and the sample again suspended in 1 ml of nano-grade

isooctane. The materials were transferred to auto-sampler vials and crimp caps applied.

Pesticide detection was done on a HP 5890 GC supplied with a Nitrogen/Phosphorus (N-P) detector and autosampler (HP 7673a). The column used was 0.25mm x 30 m SPB-5 glass capillary (Supelco, Bellefonte, Pa.). Carrier gas (linear velocity 2.46 cm^3 min^{-1}) and make-up gas (30 ml min^{-1} were high purity He. Pesticide concentration data was quantified by comparing peak area to standards of known concentration.

RESULTS

Well and site description

All sites, with the exception of well 27, were within or immediately adjacent to an active agricultural operation. Well 27, which was a community supply for a housing development in Tippecanoe county, is in excess of 1600 m from active agriculture. The initial distribution of sampling wells was random, with the 46 wells sites located in 16 different soils associations (Table 1). At 10 of these sites a second well was included. This resulted in ten well clusters or groups being established among the 25 well sites (Table 2). A cluster was defined as wells that can be grouped within a one mile radius. These clustered wells, all of which are within 400 m of each other, allow some estimation of how contamination was distributed. Well depth ranged from 4.5 to 60.9 m. Well age covers a 117 year span (1 to 118 years old). The presence and type of well head enclosure as well as the drilling methods used to establish the well, also varied (Table 3).

Approximately 49% of the wells are within 121 m of some type of animal confinement area; the majority being swine confinements. Wells 38 and 44 were inside a confinement area. No correlation (r^2 = .002) between well depth and

distance to feedlots was found (Fig. 1). This implies that in some farm operations little attention is given to the relation between water supply and possible sources of contamination. No attempt to establish the ground water flow direction or location of the farmstead septic field was undertaken.

Surface water sites

While surface water sites were randomly chosen, all sites were within 100 m of a state road and adjacent to a farm operations. Tile drain ends and indications of surface erosion were noted at some sites. Water flow was low at all sites. This is related to a lower than average rainfall for the winter of 1987.

Nutrient Contamination of Well Water

Contamination of well water by NO_3-N (to levels greater than 10 ug ml^{-1}) was confined to 4 of the 47 wells (Table 4). Contamination of well water to levels greater than 1 ug ml^{-1} occurred in 29.7 % of the wells and most frequently in wells less than 10 m deep and less than 15 m from an animal confinement area (Fig. 2). It should be noted however, that increased N levels could be found in wells distant from confinement areas if the well is shallow (less than 10 m deep).

The influence of well depth on water quality is more evident when the data is separated by county. Contamination by N in Newton county wells is limited to those less than 10 m deep (Fig. 3). This is consistent with the findings in Tippecanoe county where contamination is less than 2 ug ml^{-1} across all sites (Fig. 4). In contrast, a number of deeper wells in Jasper and Jennings county (Fig. 5 and 6) have excess NO_3-N levels. These excess levels may be related to the wells close proximity to animal confinement areas.

Lack of separation distance from the well to the confinement area appears to impact both shallow and deep wells. Well 38, a 25.9 m well located directly in an animal confinement area, consistently had elevated levels of nitrate. Wells 37 and 38 when grouped formed cluster 10, (Table 2) and provide an interesting comparison. Well 37, which is 8.5 m deep but removed by 6 m from the confinement area, showed elevated but consistently lower levels of NO_3-N. At no time did the nitrate level in well 37 exceed that in well 38. The same pattern is repeated for P. While both wells had consistently increased levels of bacteria, little difference in NH_4^+ or K was noted. This tends to imply that contamination is moving out from the confinement area to impact the adjacent well.

However, it is not clear if the same distance and depth relationship, as related to feedlots, is found in cluster 9. Well 34 and 35 are removed from the animal confinement area by greater than 30 m. Well 34 is 20 m deep as compared to 8.5 m for 35. Well 35 has a consistently elevated NO_3-N level, generally in excess of 10 ug ml^{-1}. Well 35 sits in the middle of the gravel drive way. It is our feeling that N in well 35 results from spillage of fertilizer materials or seepage from the septic field. Bacterial counts and coliforms are generally elevated in well 35.

Evaluation of Newton county well clusters 1 and 2 allows a further comparison of well placement and depth. In cluster 1, well 6 is 91 m from the confinement area and 33.5 m deep. In contrast well 7 is 3 m from the feedlot and 6 m deep. Well 7 is consistently higher in NO_3-N and K and generally had a higher bacterial count than well 6. Nitrate concentration in well 6 never exceed 0.5 ug ml^{-1} NO_3-N. Cluster 2 is comprised of two wells, 4 and 5, 6 m deep that are separated from the confinement area by 91 and 6 m, respectively. Well 5 consistently had one of the highest NO_3-N levels of any well in the

study and in contrast to all other wells, an elevated NH_4-N levels (Fig. 7). Both P and K were elevated in well 5 as compared to well 4. Well 4, while having higher than average NO_3-N levels, was always lower than well 5. The effect of well placement is more evident when clusters 1 and 2 are compared to 3. Cluster 3 is comprised of two wells greater than 24 m deep and separated from animal confinement areas by a distance of up to 1600 m. Detectable NO_3-N in both wells was consistently below 0.5 ug ml^{-1}. It seems self-evident that the production and storage of manures may be contributing to increased levels of N observed in well water. It is clear that distance to a feedlot and drilling depth impact the level of N found in well.

In contrast to the above wells, neither well in cluster 4 shows an elevated N level. However, both wells in cluster 4 are deep (30 m) and removed from animal production by a distance of 800 m. Well 44 when grouped with well 24 forms cluster 6. Cluster six is unique in that, a member of the group is immediately adjacent to an animal production facility but shows no contamination. This may reflect better handling of manures, the protection due to well depth, or lower permeability of the soils.

Wells in Tippecanoe County such as 24 generally showed little or no contamination. This results from the same combination of events that appears to protect well cluster 4 : well depth and distance from confinement area. With the exception of three, all wells studied in Tippecanoe County were greater than 15 m deep. Distances to feed lots, with the exception of well 44, were greater than 15 m.

Nutrient Contamination and Soil Association

When the contaminated wells are grouped according to soil type it is apparent that drainage may play a dominant role, especially when the well is in close proximity to animals. Contaminated Newton County wells 4 and 7 are

shallow and found in the sandy but poorly drained association of *Maumee-Newton* (Table 1.). Contaminated Jennings County wells 35, 37, 38, and 39 are deeper than 4 and 5, but are found in the well drained association of *Cincinnati-Rossmoyne-Hickory*. Of interest is Jennings County well 30. The well was continuously elevated in both NO_3-N and NH_4-N. However, the well is over 4828 m from any source of animal manure. Contamination is either from a septic system, as bacterial counts were elevated, or spillage as the well is situated near an active loading area. Again, the well is shallow in a poorly drained soil. Other contaminated wells in Jasper County, wells 18 and 19, are found in different associations (Table 1) both are in poorly drained sands.

Pesticide Contamination and Well Water

The survey of chemical use by farmers indicates that Atrazine, Bladex, Treflan, Dual, Roundup, Lorox and 2,4-D were the most frequently used materials (Table 5). Treflan use was confined to the northern counties while Atrazine, Dual, Bladex and Roundup were used extensively. The diversified spectrum of materials used in Tippecanoe County reflects the inclusion of the Purdue research farm in the study. Our analysis was confined to the Treflan, Atrazine, Dual, Lasso and Bladex.

Pesticide contamination of well water across the sample sites was not extensive. Of the approximately 400 samples collect, positive detections were limited to 8 samples (Table 6). Of the 8, only well 7 showed a repeated incident of contamination. Pesticide levels in the 6 m deep well 7 were higher than other wells exhibiting any contamination. Well 7, when grouped with well 6 formed cluster 1, was the only well where Dual appeared. The level appeared to be transient with the peak amount occurring in August. This implies a single contamination event occurred. Dual is generally considered non-leachable and contamination of this well most likely reflects activities

at the well head. Contamination by atrazine as well as alachlor, NO_3-N and K followed a similar pattern, with the peak amount occurring in August (Fig. 8). Pesticide contamination of any sort was not found in the adjacent but deeper well 6. This implies that contamination is not widespread.

Surface Water Contamination By Nutrients and Pesticides.

For the 34 surface sites that have been established, one round of sampling has been conducted. In contrast to well water, surface water are contaminated by pesticides. Of the 34 sites 20 were contaminated by some type of pesticide; most frequently recovered was atrazine. Nitrate levels were greater than 10 ug ml^{-1} at 8 sites and greater than 1 ug ml^{-1} at 26 sites. At present it is not clear if these findings are transient or a reflection of typical surface water concentrations. Further sampling to determine the contamination profile is underway.

DISCUSSION

Our findings indicate NO_3-N is the major form of contamination found in the well water of Indiana. The frequency of pesticide contamination was very low. This implies that when pesticides are handled correctly, unwanted environmental release is minimal. However, with a frequency of N contamination (NO_3-N > 1 ug ml^{-1}) greater than 29.7%, the potential for the development of human and animal health problem, is a real issue. Research has shown that young animals, particularly piglets, are susceptible to the effects of excess NO_3^- (Gehrmann-Fink and Kerber, 1978). The effects on human infants are well documented.

Pisken (1973) demonstrated the potential for feedlots to serve as sources of NO_3^- contamination for shallow well. Mielke and Ellis (1976) have shown that soils in a feedlot can average up to 90 ugsg^{-1} NO_3-N. When the entire

9.1 m profile is considered, NO_3-N levels are as high as 18,200 kg ha^{-1}. If we consider the number of farm operations in Indiana that are in some part based on animal production, roughly 86,500 (USDA 1985), and adjust for the frequency of contamination we found, a serious number of wells could be contaminated.

Factors that control the frequency of N contamination are a function of the soils physical, biological and chemical properties. Terry et al. (1981) point out that well placement and depth are important considerations. Secondly, they point out that the formation of compacted layers, as a result of animal traffic, can dramatically alter the transport process. Our findings indicate soil drainage exerts a strong affect on the degree of influence that a feedlot will have.

Nutrient movement away from a feedlot, either horizontally or vertically, is a function of the soil and its drainage character. Shaw (1982) evaluated two soils of different textures. He found that in sandy well drained soils nitrate moved evenly down the profile. In heavier textured soils nitrate, was slow to move, but disappeared once below the plow layer. Lund et al. (1974) compared NO_3-N movement in 15 soils of varying texture. They found that soil texture could explain 86% of the variability in NO_3-N movement. Of the components that makeup texture, clay content was the most significant factor. Increased clay content resulted in decreased infiltration and increased retention of NO_3-N in the upper profile.

While it is difficult to draw exact conclusions based on wells where the use is not constantly controlled (monitoring wells), it is clear that when the well is shallow and the contamination is retained in the upper portion of the profile increased NO_3-N in the water will result. In better drained soils,

the contamination affects both shallow and the deeper wells if they are supplied with a leachable N source.

King et al. (1985) have shown that when high rates of animal effluent are applied to permeable soils, NO_3-N levels can be increased up to 50 ug g^{-1}soil at 120 cm depth. However, they could only account for up to 55% of the applied N when coring to this depth. They concluded that the rest was lost to leaching. This implies that the N leaching from our feedlots is intercepted by the water bearing zone, brought up by the supply well, and acts as an indicator of the degree of deep percolation. However, without knowing the aquifer's size, placement and direction of flow, exact conclusions on aquifer concentration are not possible.

Of the 47 farm supply wells studied, 49% were within 121 meters of an animal confinement area. Of the 23 wells in this category 43% had elevated N levels. Of the 24 wells outlying the confinement areas by distances greater than 121 m, 16 % had elevated N levels. Terry et al. (1981) reported that N levels decrease with distance from large storage lagoons. They showed that at distances of up to 103 m, shallow (7.6 m) wells in a poorly drained soil were impacted by NO_3. However, a well of 21 m depth at the same location was not influenced. Our clustered wells showed that in a typical farm operation, less distance is needed to see a decreased N level. It is apparent that separation distances (either horizontal or vertical) offer some effective protection for well water. The separation distance is a function of the confinement system and soil type. Like Terry et al. (1981) our shallow wells in slowly draining soil were more affected at greater distances than were the deeper wells. Our lessened distances reflect the fact that Terry et al. (1985) were dealing with large impoundments of liquid waste. In our situation, waste is surface applied and left to dry.

While we are not able to directly separate fertilizer source N from the waste source N, the suggestion that fertilizer N is responsible for an increased nitrate level in farm wells of the region is not supported by our findings. However, when the well is separated from the feedlot and NO_3-N levels are increased, fertilizer N may be a major factor. It is our feeling that spillage at or near the well head may be the chief source of fertilizer based NO_3-N. This suggestion is based on interviews with farmers and the overall levels of N observed in the regions' ground water. If general fertilizer application was serving as a source of N then this coupled with the year round N loading that occurs from feedlots should effectively increase the back ground levels. Overall, levels of N in the well water of the 4 counties were not significantly higher and contamination appeared to be of a point source. The lack of an overall increased N level in the ground water of the regions suggest that active large scale inputs are not occurring.

Pesticide analysis indicated, similarly to the results found for NO_3-N levels, that widespread contamination was not occurring. If well 7 is removed from consideration, pesticides were detected in only 7 of approximately 400 water samples. When well 7 is considered, our results parallel Lym and Massersmith (1988) findings for a study of picloram contamination in the waters of North Dakota. They reported that the incidence of well contamination to be low, but when present, the pesticide introduction could be traced to a particular event or spill. Further, as in our findings they recovered more materials in the surface waters than in well water for the studied regions.

It is our feeling that the increased level of pesticides found in the surface waters of the northern portion of the state may reflect inputs from field drain tile lines. It is also our feeling that the very high levels

found in the southern portion of the state reflect intentional dumping into the ditch.

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Table 1. Well number, soil association and drainage for sites under study.

Well No.	Soil Association/Drainage†
	Newton County
1	Odell-Chalmers/poorly drained in glacial till.
2,10,11,12,42	Rensselaer-Darroch/poorly drained in lake deposited sand and silt.
3	Parr-Miami/well drained in glacial till.
4,5,6,7,8,9	Maumee-Newton/poorly drained in lake deposited sand or outwash.
	Jasper County
13,14,15	Maumee-Gilford-Rensselaer/poorly drained in outwash or lake-deposited sand and silt.
16	Parr-Miami/well drained in glacial till.
18	Maumee-Newton/poorly drained in lake deposited sand or outwash.
19	Rensselaer-Darroch/poorly drained on outwash or lake-deposited sand and silt.
	Tippecanoe County
20,21,26,49 50,51	Raub-Ragsdale/poorly drained in glacial till or wind blown silts.
22,54,55,56	Odell-Chalmers/poorly drained in glacial till.
23,25,27,40	Fincastle-Ragsdale-Brookston/poorly drained in silts or glacial till.
24,44,52	Sidell-Parr/poorly drained in glacial till
	Jennings County
30	Avonburg-Clermont/poorly drained with fragipans, in wind-blown silts or weathered glacial till.
31	Genesee-Shoals-Eel/well drained in alluvial deposits.
32,34,35,36 37,38,39,43	Cincinnati-Rossmoyne-Hickory/well drained in weathered till.
33	Wakeland-Stendal-Haymond/moderate drainage in acid alluvail till.

† Taken from Purdue University Cooperative Extension Bulletins AY-50-56, 37, 79, and 40 for Newton, Jasper, Tippecanoe and Jennings Counties, respectively.

Table 2. Well cluster† groupings, depth, distance to confinement areas and ranges of NO_3-N and NH_4-N.

Well No.	Depth M	Distance M	Range of NO_3-N ug ml^{-1}	Range of NH_4-N ug ml^{-1}
Newton				
Cluster 1				
6	33.5	91.48	0 - 0.38	0.66-1.20
7	6.0	3.0	2.5 - 5.7	0.22-0.76
Cluster 2				
4	6.0	91.48	4.0 - 6.8	0 -0.38
5	6.0	15.2	21.3 -24.4	3.7 -6.72
Cluster 3				
10	24.3	1207	0 - 0.49	0.16-0.93
42	36.5	1609	0.05- 0.44	0.49-1.14
Jasper				
Cluster 4				
13	30.4	804	0.27- 2.46	0.11-1.91
14	30.4	804	0.11- 0.33	0.38-0.6
Tippecanoe				
Cluster 5				
21	34.7	121	0.05- 5.13	0.05-0.87
26	30.4	402	0.22- 0.55	0.27-1.53
Cluster 6				
24	36.5	45.7	0.05- 3.88	0.33-0.66
44	60.9	0	0.05- 0.71	0.05-0.87
Cluster 7*				
54	25.9	4828	0.16- 0.22	0.38-0.55
55	15.2	4828	0.22- 0.27	0.33
56	6.0	4828	0.33- 0.77	0.33-0.44
Jennings				
Cluster 8				
31	15.2	1609	0.11- 0.60	0 -0.49
32	18.2	1609	0.55- 1.09	0.05-1.04
Cluster 9				
34	20.7	24.3	0.16- 0.60	0.49-0.98
35	8.5	30.4	12.29-13.92	0.16-0.49
Cluster 10				
37	8.5	6.09	0.27- 6.01	0.11-0.55
38	25.9	0	2.24-12.25	0.11-0.55

† Well sites are within a one mile radius.
* Range based on two samplings.

Table 3. Well number, depth, distance to confinement area, age and existence of surface housing.

Well No.	Depth M	Distance M	Age Yr.	Housing Y/N
		Newton		
1	18.2	1609	118	y
2	12.8	30.4	10	n
3	44.5	30.4	---	y
4	6.0	91.4	60	n
5	6.0	15.2	7	n
6	33.5	91.4	12	y
7	6.0	3.04	40	y
8	6.0	45.7	26	n
9	7.0	1609	1	n
10	24.3	1207	50	y
11	26.2	60.9	12	y
12	28.6	4828	77	n
42	7.6	1609	16	y
		Jasper		
13	30.4	804	50	n
14	30.4	804	50	n
15	35.6	15.2	23	y
16	53.6	4828	22	y
17	----	----	---	-
18	4.5	804	13	n
19	12.8	2.4	26	y
		Tippecanoe		
20	50.0	91.4	27	y
21	34.7	121.9	50	n
22	38.4	91.4	35	y
23	----	4828	32	y
24	36.5	45.7	40	y
25	27.4	1609	38	y
26	30.4	402	65	y
27	53.3	3218	30	y
40	35.6	1609	1	y
44	60.9	0	60	y
49	6.0	1609	25	y
50	18.2	121.9	3	n
51	60.9	1609	18	n
52	24.3	27.4	18	n
53	----	----	---	-
54	25.9	4828	50	y
55	15.2	4828	85	y
56	6.0	4828	50	n
57	----	----	---	-

Table 3. continued

Well No.	Depth M	Distance M	Age Yr.	Housing Y/N
		Jennings		
30	4.8	4828	1	y
31	15.2	1609	59	y
32	18.2	1609	---	n
33	22.8	60.9	20	y
34	20.7	24.3	40	y
35	8.5	30.4	70	y
36	24.3	6.0	25	y
37	8.5	6.0	100	y
38	25.9	0	60	y
39	7.6	4828	13	y
43	31.4	244	10	y

Table 4. Well number and average level of NO_3-N, NH_3-N, P, and K, occurrence of pesticides in well samples.

Well No.	NO_3-N	NH_3-N	P	K	Pesticide
	ug ml^{-1}				+/-
		Newton County			
1	0.56	0.29	.013	7.07	
2	0.28	0.63	.010	1.39	
3	0.27	0.62	.007	3.32	
4	6.06	0.21	.015	10.0	
5	23.1	5.00	.102	14.0	
6	0.17	0.97	.009	3.9	
7	3.66	0.44	.007	11.6	+
8	0.35	0.50	.019	0.64	
9	0.79	0.44	.005	1.84	
10	0.35	0.58	.063	3.16	
11	0.26	0.81	.011	2.82	
12	0.21	0.75	.015	2.75	
42	0.26	0.74	.021	2.92	
		Jasper County			
13	0.62	0.61	.043	2.67	
14	0.24	0.48	.047	4.36	
15	0.34	1.78	.100	1.93	
16	0.41	1.76	.042	9.98	
17	0.73	0.27	.004	2.07	
18	5.74	0.32	.032	1.76	
19	22.61	0.26	.002	17.0	
		Tippecanoe County			
20	1.65	0.22	.020	0.97	
21	1.09	0.57	.009	3.54	
22	0.23	0.81	.008	2.46	
23	2.64	0.26	.006	2.45	
24	0.86	0.49	.009	3.02	+
25	0.29	0.73	.010	2.72	+
26	0.32	0.53	.014	2.37	+
27	0.34	0.25	.003	2.27	
40	0.27	0.22	.009	1.26	
44	0.32	0.44	.004	1.52	+
49	1.49	0.55	.008	1.29	
50	0.29	0.81	.008	1.72	+
51	0.38	0.45	.005	0.99	
52	0.21	0.37	.004	1.03	
53	0.31	0.44	.009	2.70	
54	0.19	0.47	.010	1.47	
55	0.25	0.33	.005	2.33	
56	0.55	0.39	.008	1.12	

Table 4. continued

Well No.	NO_3-N	NH_3-N	P	K	Pesticide +/-
	ug ml^{-1}				
			Jennings County		
30	11.17	0.30	.004	4.67	
31	0.32	0.30	.004	0.68	+
32	0.81	0.39	.011	1.38	
33	0.36	0.87	.020	2.78	
34	0.30	0.71	.007	2.75	
35	12.95	0.38	.034	1.85	
36	0.26	0.42	.008	1.11	
37	4.44	0.28	.014	2.41	
38	7.37	0.31	.052	1.98	
39	2.48	0.42	.016	1.83	
43	0.52	0.54	.012	1.07	

Table 5. Chemicals used by farmers participating in study.

CHEMICAL	Total	Distribution of Chemical Use			
		Newton	Jasper	Tippec.	Jennings
Atrazine	35	9	5	12	9
Bladex	22	7	5	6	4
Treflan	22	5	0	9	8
Dual	19	6	3	9	2
Roundup	21	6	2	7	6
Lorox	12	2	4	1	5
2,4-D	10	1	4	3	1
Lasso	9	4	2	3	0
Carbofuran	6	2	0	3	1
Dicamba	6	3	1	2	0
Sencor	4	1	0	2	1
Ramrod	3	1	0	1	1
Command	2	0	1	1	0
Scepter	2	0	0	1	1
Sutan	4	1	0	1	2
Eradicane	1	0	0	0	1
Preview	1	0	0	0	1
Sonalon	1	0	1	0	0
Counter	2	0	0	2	0
Simazine	1	1	0	0	0
Poast	1	0	0	1	0
Basagram	1	0	0	1	0
Cobra	1	0	0	1	0
Classic	1	0	0	1	0
Tackle	1	0	0	1	0

Table 6. Pesticide detection in well samples for the 4 counties.

Well No.	Tref.	Atra.	Alac.	Metol.	Cyan.
	---------- ug l^{-1} ----------				
7	0	0.4 -2†	1.2 -4.6†	5.4-34.2†	0
24	0	0.5*	0	0	0
25	0	0	0.44	0	0
26	3.36*	0.52	0	0	0
30	0	0.34	0	0	0
31	0	0	68.4	0	0
44	6.94	1.58	0	0	0
50	0	0	0	0	2.04

† Indicates multiple detection events and range of values.

* Indicates a single detection event.

Table 7. Pesticide detections† in surface water samples for the four counties.

Site No.	Date	NO_3-N	Tref.	Atra.	Alac.	Meto.
		ug ml^{-1}	----------	ug l^{-1}	----------	
			Jennings County			
101	5/10	1.6	0	0	0	0
102		11.3	0	0	0	0
103		0.3	0	4.16	10.3	0
104		0.7	0	4.76	0	0
105	5/10	2.5	0	0	0	0
106		0.3	0	0	0	0
107		16.7	0	1.68	0	0
108		0.3	2.14	0	76.74	41.42
			Newton County			
109	5/18	14.2	0	0	0	0
110		0.2	0	0.82	1.20	0
111		0.4	0	0.90	1.32	0
112		0.6	0	0	0	0
113		0.3	0	0	0	0
125	5/31	28.0	0	0.38	0	0
126		20.5	0	0.38	0	0
127		12.3	0	0.60	0	0
128		4.0	0	0.64	0	0
			Jasper County			
114	5/18	0.4	0	0.30	0	0
115		0.7	0	0	0	0
116		12.1	0	0.40	0	0
117		6.1	0	0.26	0	0
129	5/31	7.1	0	0.68	0	0
130		7.9	0	0.56	0	0
131		1.9	0	1.36	0	0
132		1.0	0	0	0	0
133		0.9	0	0.40	0	0
134		0.4	0	.740	0	0
			Tippecanoe County			
118	5/25	0.8	0	0	0	7.30
119		2.0	0	0	0	0
120		4.8	0	0	0	0
121		1.1	0	0	0	0
122		3.4	0	0	0	0
123		5.7	0	0	0	0
124		9.4	0	0	0	0
135	6/06	5.5	0	0	0	0
136		1.8	0	0	0	0
137		13.6	0	0	0	0
138		14.4	0	0	0	0
139		9.8	0	0.08	0	0

† One round of sampling. Tests are on-going.

FIGURE LEGENDS

Fig. 1. Relationship of well depth to distance from confinement areas for wells at less than 125 m separation distance.

Fig. 2. Relationship of well depth, distance of separation from feedlot and average NO_3-N concentration.

Fig. 3. Average NO_3-N concentration by depth of well for Newton sites.

Fig. 4. Average NO_3-N concentration by depth of well for Tippecanoe sites.

Fig. 5. Average NO_3-N concentration by depth of well for Jasper sites.

Fig. 6. Average NO_3-N concentration by depth of well for Jennings sites.

Fig. 7. Relationship of well depth, distance of separation from feedlot and average NH_4-N concentration.

Fig. 8. Concentration of profile NO_3-N, K, Dual, Atrazine and Lasso in well 7.

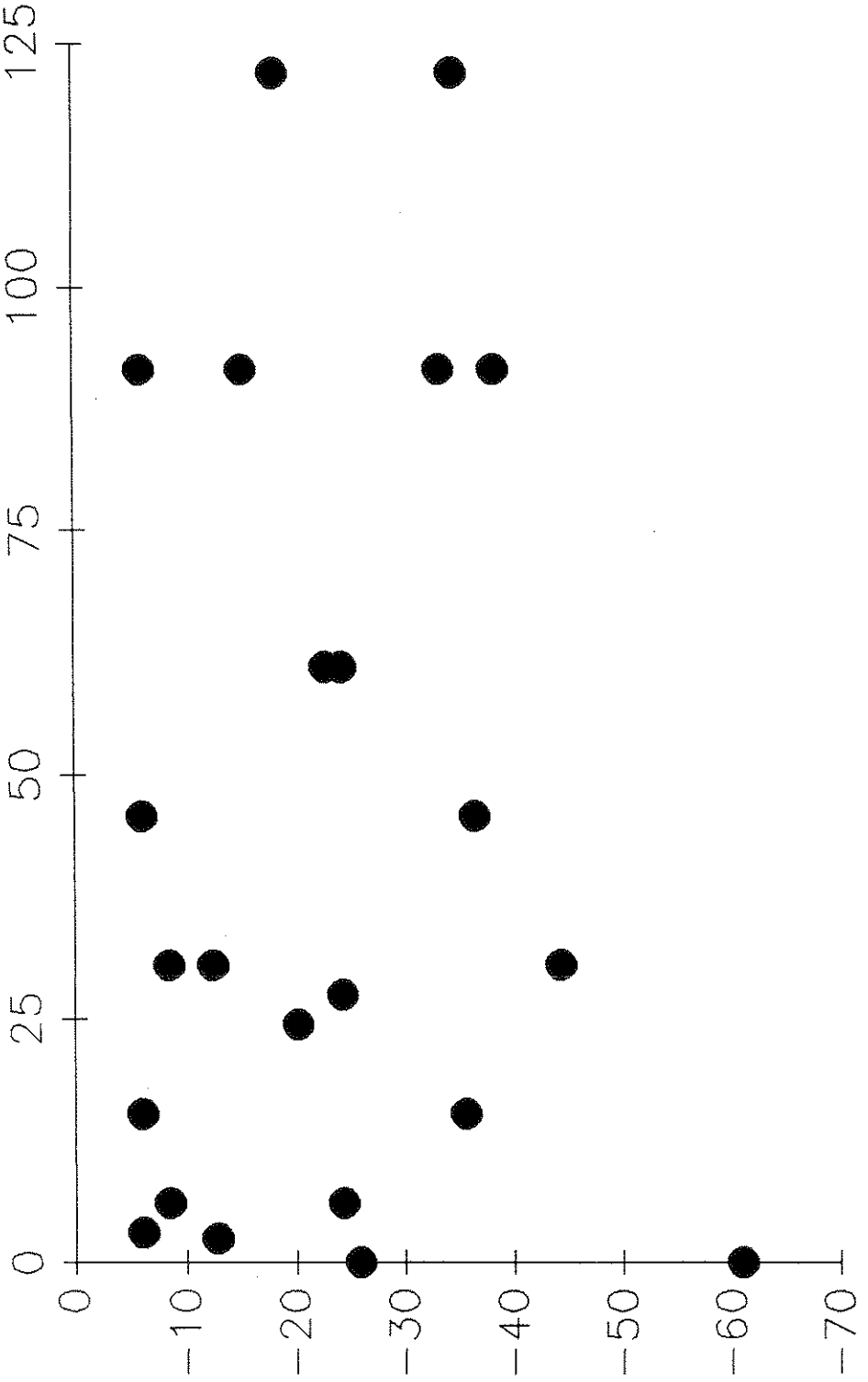

Distance to Feedlot (m)
0
25
50
75
100
125
Well Depth (m)
0
-10
-20
-30
-40
-50
-60
-70

Fig. 1

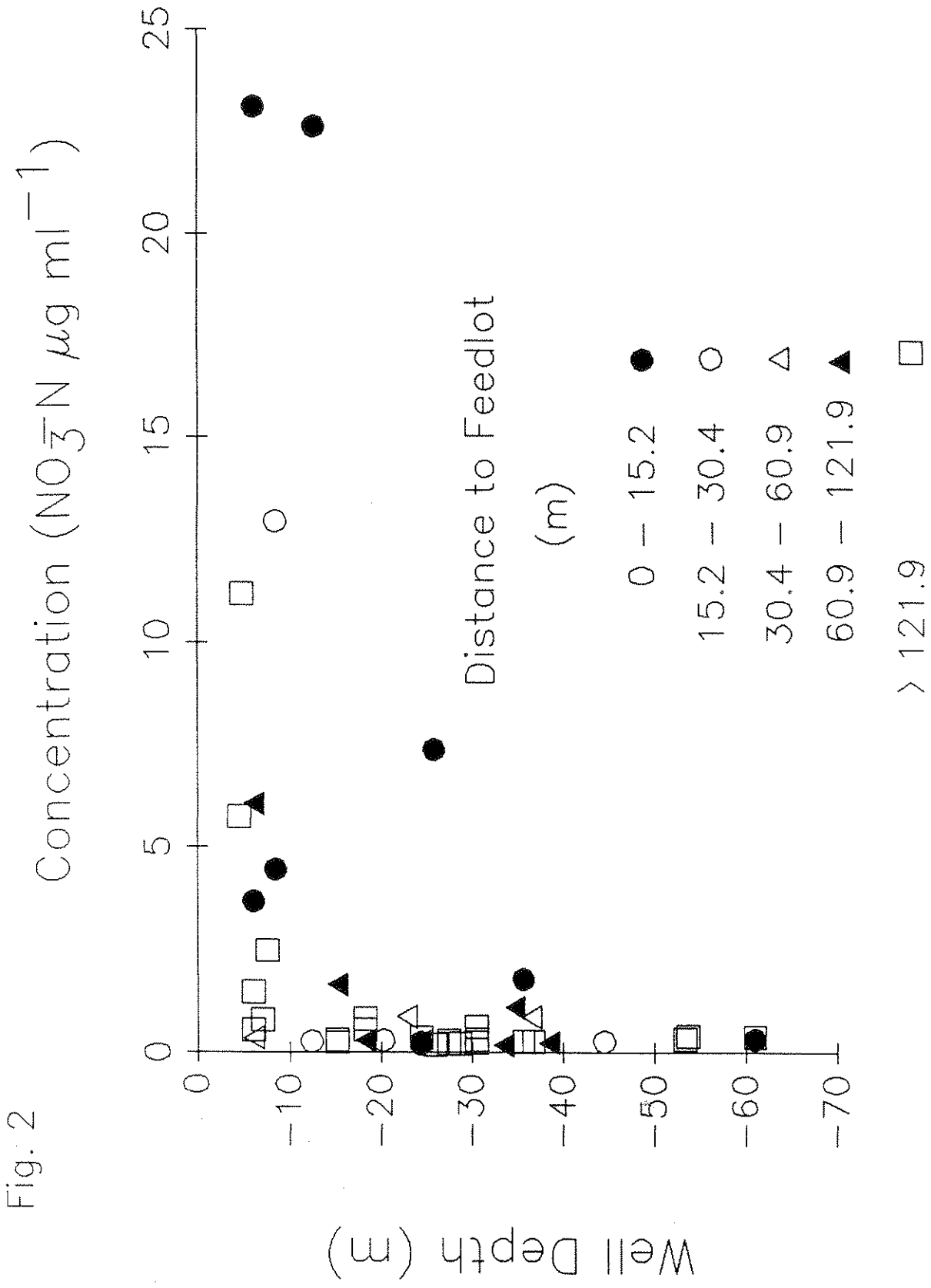

Concentration (NO_3^-N μg ml^{-1})
0
5
10
15
20
25
Well Depth (m)
0
−10
−20
−30
−40
−50
−60
−70
Distance to Feedlot
(m)
0 − 15.2
15.2 − 30.4
30.4 − 60.9
60.9 − 121.9
> 121.9

Fig. 2

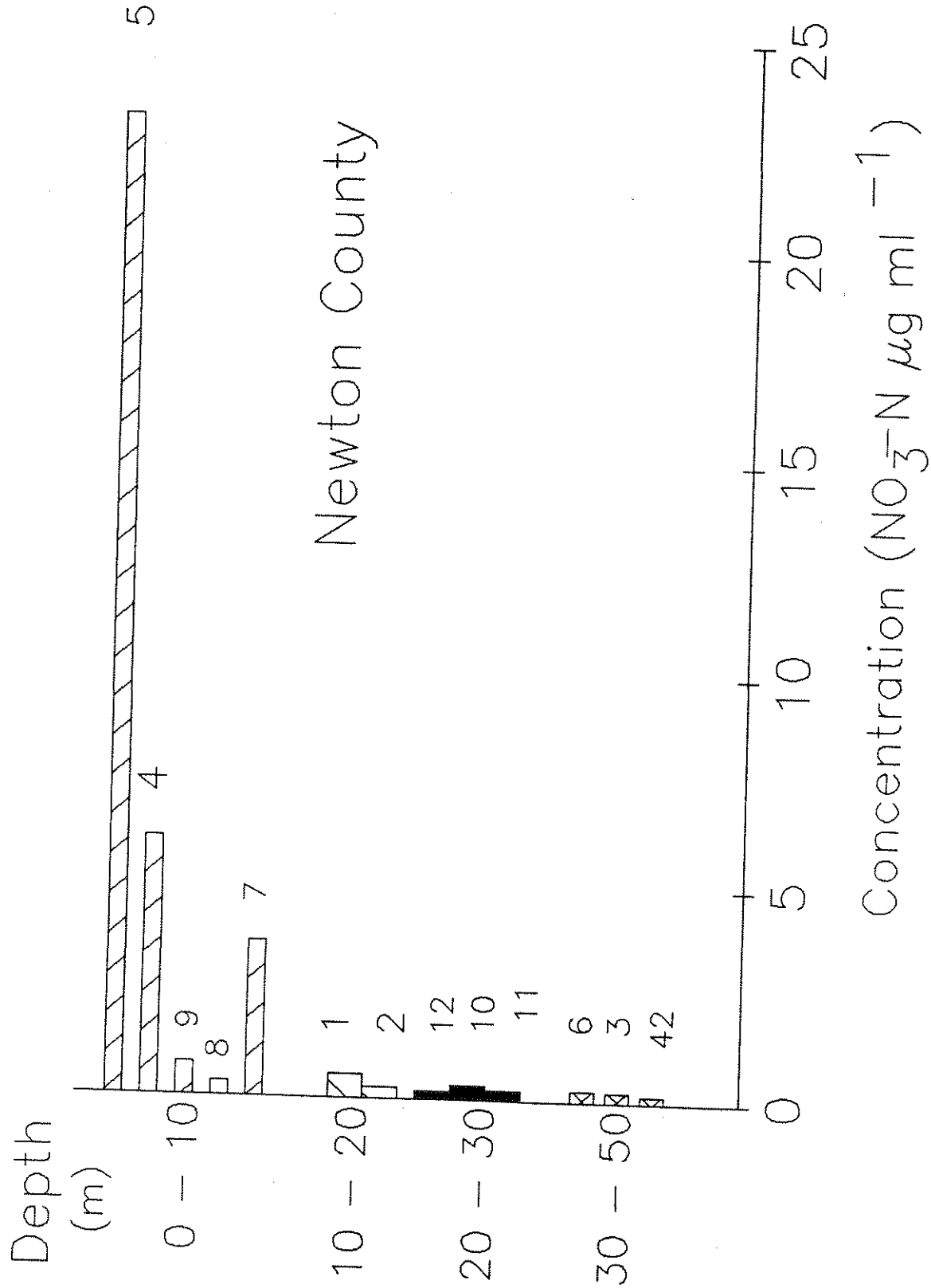

Newton County
Depth (m)
0 – 10
10 – 20
20 – 30
30 – 50
5
4
9
8
7
1
2
12
10
11
6
3
42
0
5
10
15
20
25
Concentration (NO_3^-N μg ml^{-1})

Fig. 3

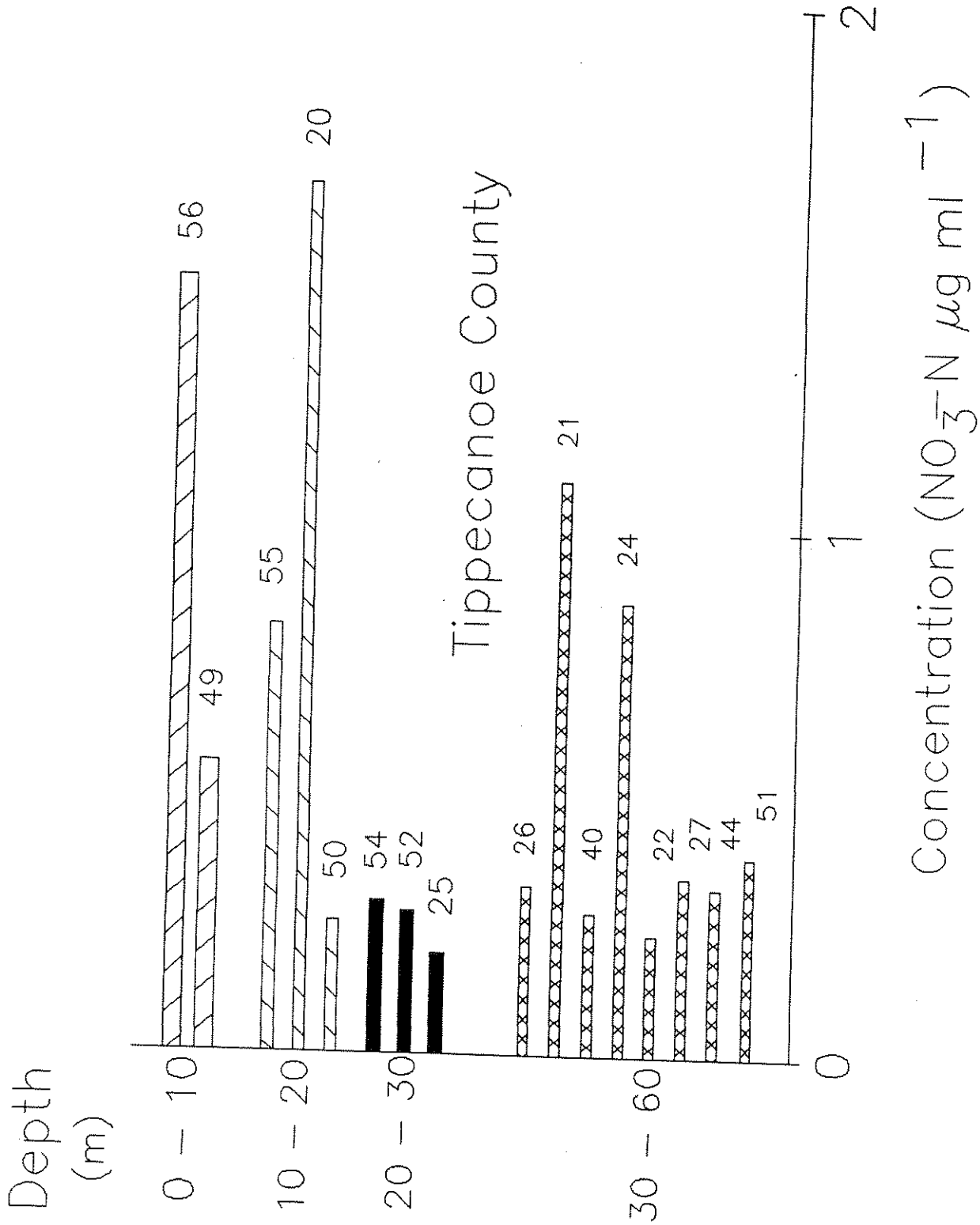

Depth
(m)
0 – 10
10 – 20
20 – 30
30 – 60
56
49
55
20
50
54
52
25
26
21
40
24
22
27
44
51
Tippecanoe County
0
1
2
Concentration (NO_3^-–N $\mu g\ ml^{-1}$)

Fig. 4

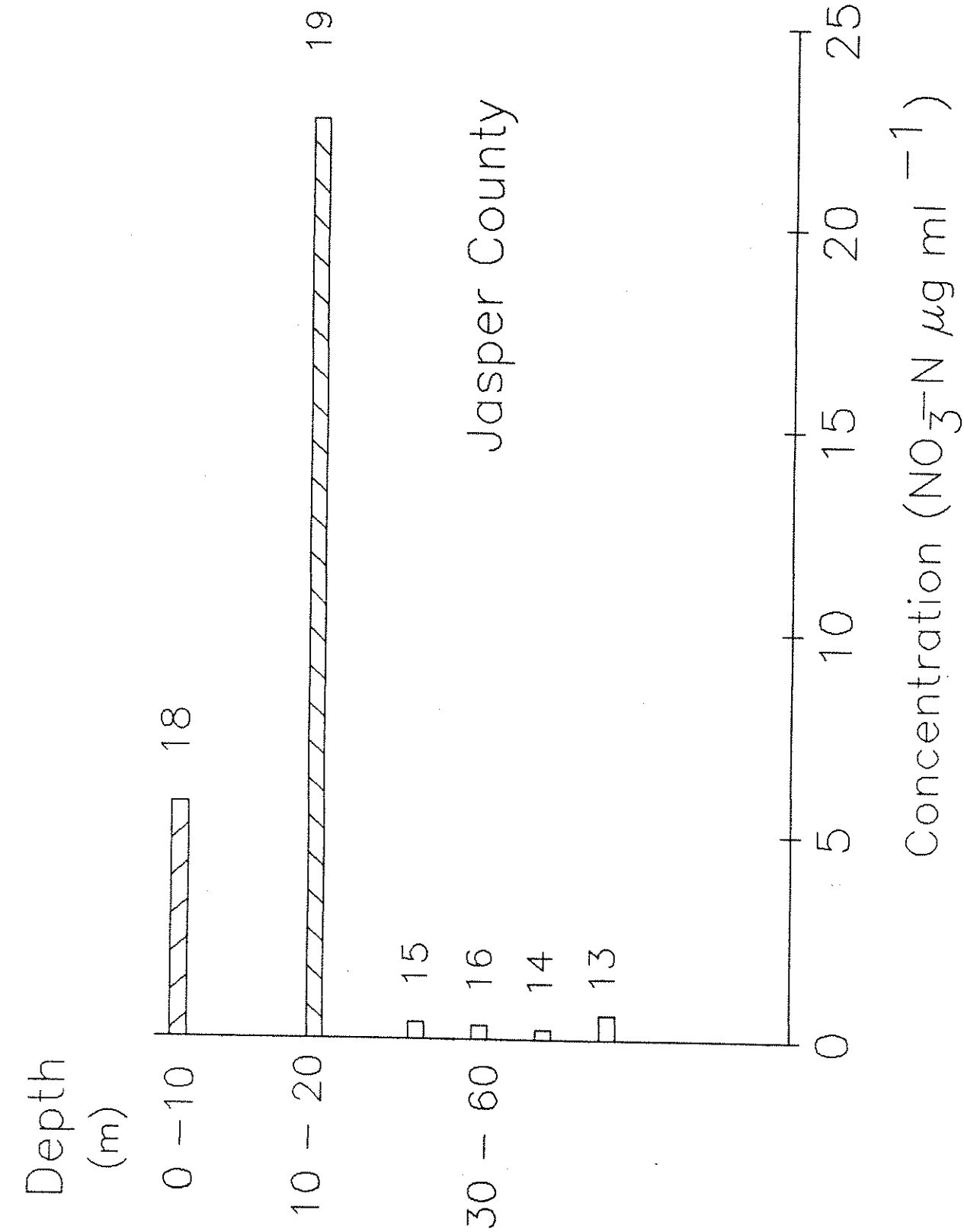

Jasper County
Depth (m)
0 – 10
10 – 20
30 – 60
18
19
15
16
14
13
0
5
10
15
20
25
Concentration (NO_3^--N $\mu g\ ml^{-1}$)

Fig. 5

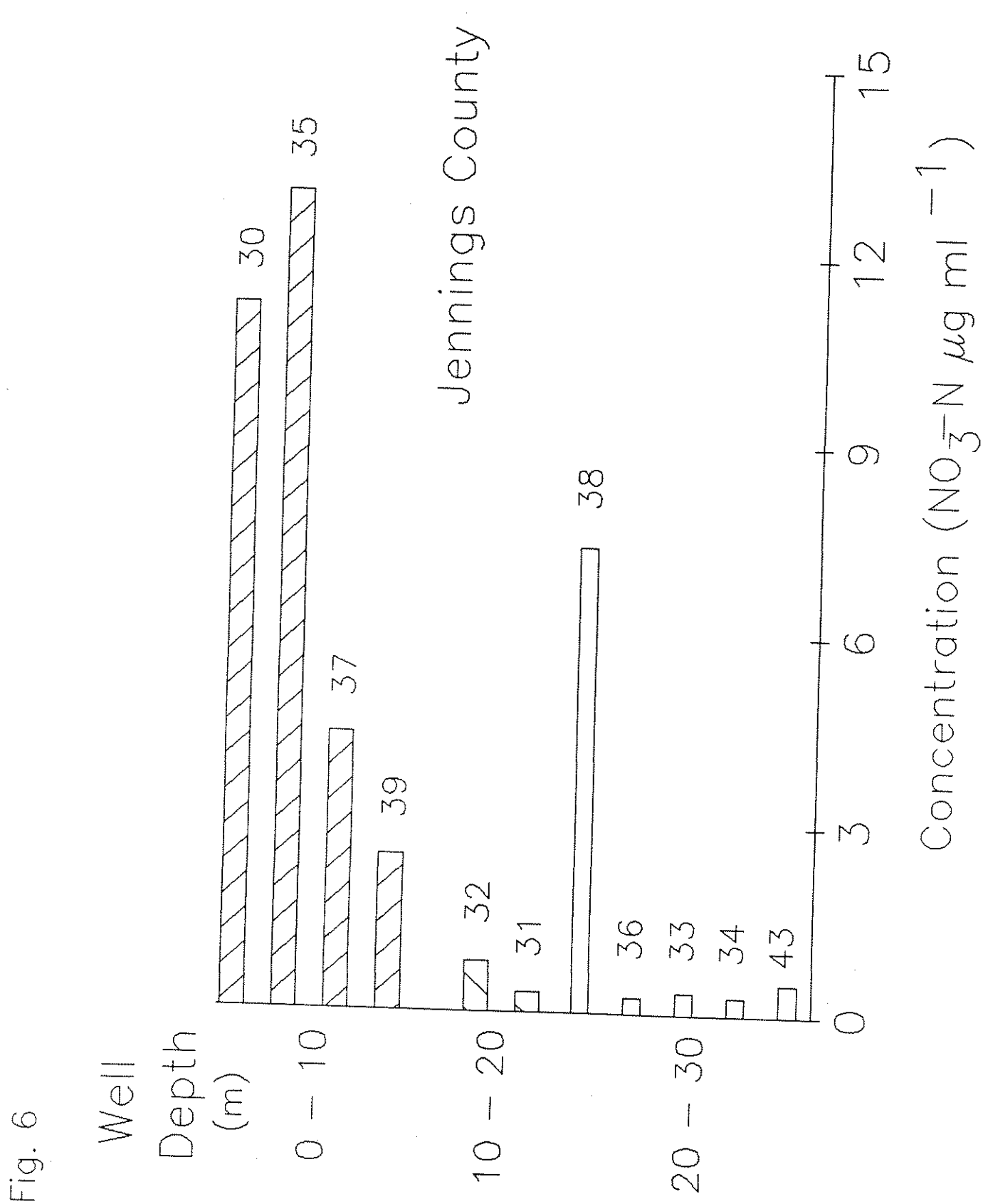

Jennings County
Well Depth (m)
0 – 10
10 – 20
20 – 30
30
35
37
39
32
31
38
36
33
34
43
0
3
6
9
12
15
Concentration (NO_3^-–N $\mu g\ ml^{-1}$)

Fig. 6

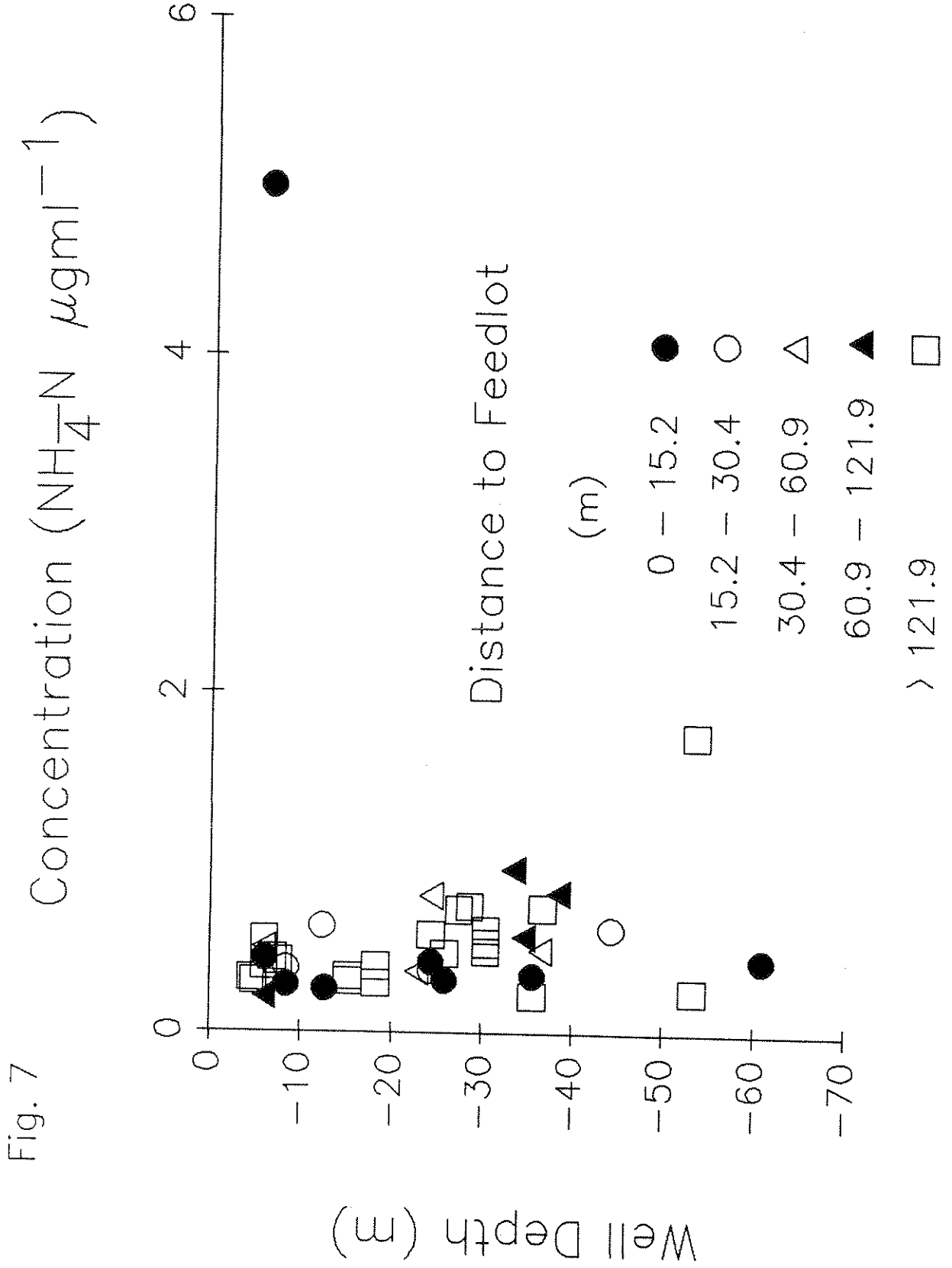
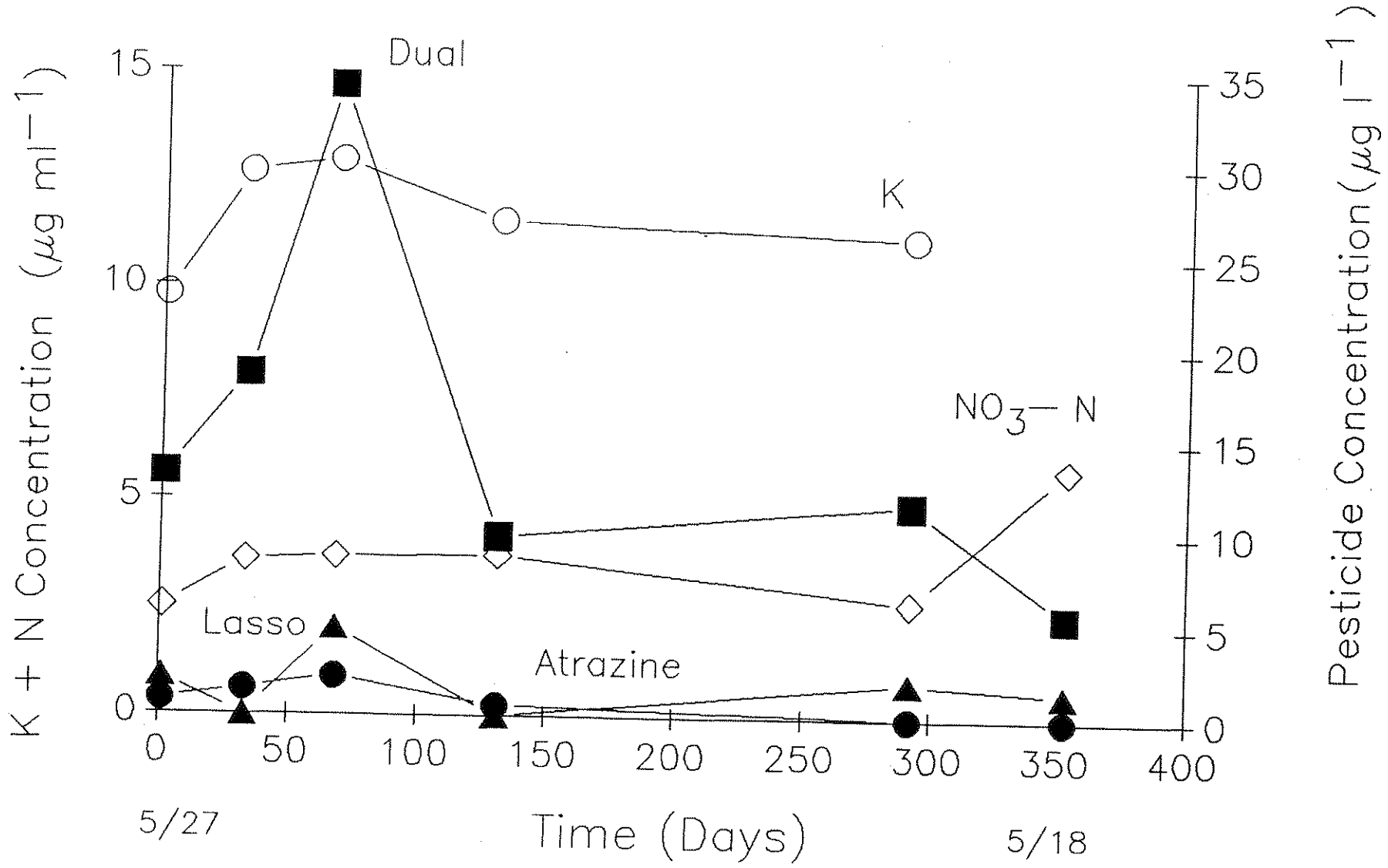

Concentration (NH_4^+N $\mu g ml^{-1}$)
0
2
4
6
Well Depth (m)
0
−10
−20
−30
−40
−50
−60
−70
Distance to Feedlot
(m)
0 − 15.2
15.2 − 30.4
30.4 − 60.9
60.9 − 121.9
> 121.9

Fig. 7

Fig. 8


Dual
K
NO_3— N
Lasso
Atrazine
K + N Concentration (μg ml^{-1})
Pesticide Concentration (μg l^{-1})
Time (Days)
5/27
5/18
0
5
10
15
0
50
100
150
200
250
300
350
400
0
5
10
15
20
25
30
35

PART II

INTRODUCTION

Anilines are used in the manufacture of pesticides, dyes, plastics, and pharmaceuticals (Kearney and Kaufmann, 1975; Meyer, 1981). They are also formed during microbial transformation of nitroaromatic compounds such as explosives, dinitroaniline herbicides such as Treflan and Prowl, and nitropyrenes (Hallas and Alexander, 1983; Kinouchi and Ohnishi, 1983; McCormick et al., 1976). Thus, anilines are widely distributed in the environment, especially in soils commonly receiving herbicide applications. In Indiana it is estimated that in 1987 some 2.65×10^5 ha of soil was treated with the aniline compound Treflan [N,N-dipropyl-4-(Trifluoromethyl)-2,6-dinitroaniline]. In principle, a variety of physicochemical processes may affect the environmental fate of anilines -- these include evaporation, photooxidation, and chemical binding (Bollag et al., 1978). In aquatic environments, Lyons et al. (1984) showed that biodegradation was the most significant mechanism of aniline removal. In soils, mineralization of anilines may occur slowly (Suss et al., 1978); chemical transformations in which anilines are incorporated into humic compounds (Bollag et al., 1978), or react to form recalcitrant molecules such as azobenzenes and triazenes (Kearney et al., 1969; Minard et al., 1977) can occur.

Pure cultures of microorganisms which can degrade aniline, or in some instances chlorinated anilines (Zeyer et al., 1985), have been isolated. Aniline was metabolized by oxidative deamination to catechol; the aromatic ring was then cleaved via either the ortho or meta pathway (Bachofer et al., 1975; Zeyer et al., 1985; Anson and MacKinnon, 1984). Aniline catabolism had to be induced by exposure to aniline in all aniline degrading microbes. However, in the pseudomonad SB3, catechol 2,3-oxygenase was a constitutive

enzyme, whereas the oxidative deamination of aniline to catechol was inducible (Kaminski et al., 1983).

In natural environments, microbes which degrade aniline will also encounter other organic energy substrates. Although these other substrates may lead to higher biomass levels which then catalyze faster rates of aniline transformation, these alternative substrates may also inhibit aniline metabolism at the cellular level. For example, in Pseudomonas multivorans Anl, carbohydrates, organic acids, and amino acids were used in preference to aniline in mixed-substrate cultures (Helm and Reber, 1979).

In this report, we describe a bacterium which grows relatively rapidly on aniline and in which aniline catabolism is not repressed by the presence of other readily utilized organic substrates.

MATERIALS AND METHODS

Bacteria were grown using XBM mineral salts, which contained 10 mM phosphate buffer (pH 7), 0.25 mM $MgSO_4$, 10 mm NH_4Cl, 5 micromolar $CaCl_2$, 15 micromolar $FeCl_3$, 23 micromolar disodium ethylenediaminetetraacetate, and 25 micromolar sodium citrate. In addition, XBM contained 1 ml of SL7 trace element solution (Biebl and Pfennig, 1981) per liter. In general, organic substrates were added at concentrations providing 50 mmol C per liter. Cultures were incubated at 30°C. Growth was monitored by measuring the optical density at 600 nm on a Gilford 250 spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio). An optical density of 1.0 corresponded to 1.4×10^9 cells/ml.

The bacterial isolate was characterized by morphological and biochemical tests as described in Gerhardt (1981). Chemoautotrophic growth on H_2 was tested as described in Konopka and Szentes (1984).

Uptake of [^{14}C]-aniline was determined by measuring both ^{14}C incorporated into particulate material and ^{14}C respired as $^{14}CO_2$. Uniformly labeled [^{14}C]-aniline (Sigma Chemical Company, St. Louis, Missouri; specific activity = 13.62 mCi/mmol) was added to 5 ml suspensions of cells (7 x 10^6 cells/ml) that had been washed and resuspended in XBM salts. For concentrations above 0.75 micromolar, unlabeled aniline was also added to achieve the desired concentration. The samples were incubated for 20 min at 30°C in 25 ml flasks sealed with red butyl rubber septa. At the end of the incubation period, 0.2 ml of 1 M HCl was injected, and 0.1 ml of phenethylamine was added to a polypropylene trap that had been placed above the liquid in the flask. Samples were incubated for 24 h to allow $^{14}CO_2$ to be absorbed into the phenethylamine traps. The aqueous sample was then filtered through 0.45 μm filters (Gelman GN-6, Gelman Sciences Inc., Ann Arbor, Michigan). Filters and trapping solution were counted after adding ACS liquid scintillation fluid (Amersham Corp., Arlington Heights, Illinois). Data from experiments on uptake rates as a function of aniline concentration were fitted to the Michaelis-Menten equation by using the NLIN procedure of the SAS system (SAS Institute Inc., Cary, North Carolina).

The stimulation of oxygen consumption by organic substrates in washed, resting cell suspensions was measured using a Clark oxygen electrode (Yellow Springs Instruments, Yellow Springs, Ohio). Two ml of washed cells (3 x 10^8 cells/ml) were incubated at 30°C, and the rate of endogenous respiration monitored. Test substrate was then added at a final concentration of 1 mM, and the rate of oxygen consumption was monitored. Rates were corrected for endogenous respiration.

Catechol oxygenase activities were measured in cell extracts of log phase cells. Cells were harvested by centrifugation, washed in XBM salts, and

finally resuspended in 10 mM phosphate buffer (pH 7). Cells were broken by sonication (Sonifier Cell Disruptor model W185, Heat Systems-Ultrasonics Inc., Plainview, New York). The extract was centrifuged at 25,000 x g for 15 min, and the supernatant was used for enzyme assays. All procedures prior to the enzyme assay were conducted at <5°C. Catechol-2,3-oxygenase activity was measured as described by Sala-Trepat and Evans (1971). Catechol-1,2-oxygenase was assayed by the method of Hayaishi et al. (1957).

The ability of substituted anilines to induce the capacity for aniline oxidation was tested as follows. Strain K1 was grown in XBM + 16 mM lactate to a density of 6 x 10^7 cells/ml. Putative inducers were added at a concentration of 1 mM, and the cultures were incubated for 3 h. The cells were centrifuged, washed, and resuspended in XBM salts at a density of 7 x 10^6 per ml. The rate of [^{14}C]-aniline metabolism at a concentration of 10 micromolar was determined as described above.

Aromatic chemicals were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin) and were used as is.

RESULTS

Strain isolation and characterization. Strain K1 was isolated from a raw sewage collected at the West Lafayette, Indiana, sewage treatment plant. The sample was inoculated into a batch culture of XBM mineral salts containing 8 mM aniline and incubated at 25°C. After several transfers, a pure culture was isolated on agar medium containing XBM + 8 mM aniline.

Strain K1 is a Gram-negative, motile rod that is catalase and oxidase positive. Carbohydrates (glucose, xylose, ribose, maltose, and lactose) were not used as growth substrates. Growth did occur on fructose, the organic acids acetate, lactate, and succinate, as well as on the alcohols methanol,

ethanol, and glycerol, and the amino acids alanine, phenylalanine, and tryptophan. Strain K1 could grow chemoautotrophically on H_2 and CO_2. This isolate did not fix N_2. The organism did not grow under anaerobic conditions, even in the presence of nitrate. On the basis of these characteristics, strain K1 was characterized as a Pseudomonas.

Growth and aniline metabolism. Strain K1 grew in a minimal salts medium + 8 mM aniline with a generation time of 2.2 h at 30°C (Fig. 1). The organism grew in media containing 32 mM aniline but not at a concentration of 64 mM. The growth rate on aniline was as high as on any substrate tested. For example, the doubling time with lactate as carbon source was 2.3 h, and with acetate it was 3.2 h.

The kinetics of aniline uptake by cells grown on 8 mM aniline was determined using [^{14}C]-aniline (Fig. 2). Production of $^{14}CO_2$ and incorporation into particulate material was monitored. During the 20 min incubations, about 85% of the metabolized aniline was converted to CO_2. Nonlinear regression of the data to fit the Michaelis-Menten model resulted in a half-saturation constant of 3.8 micromolar and a V_{max} of 4.5 micromoles/L/h for suspensions containing 7 x 10^6 cells/mL.

Aromatic substrate range. The range of aromatic substrates used by K1 was tested in two ways. (1) The substrates were provided as sole sources of carbon and energy for growth in minimal media. (2) The substrates were added to washed suspensions of cells that had been grown on aniline, to determine if they stimulated respiration.

The only aromatic compounds other than aniline that supported growth of K1 were the amino acids tryptophan and phenylalanine (Table 1). In addition, no growth was found on any isomer of dihydroxybenzoate or on pyrogallic acid.

Respiration by washed suspensions of aniline-grown cells was strongly stimulated by catechol (Table 1). A few aminoaromatics supported respiration rates 5 - 30% of that found for aniline, but most substituted anilines and all other aromatic compounds did not stimulate respiration.

Aniline-grown cells did respire the organic acids acetate and lactate at low rates (Table 2). However, when K1 was grown on either lactate or acetate, respiration was not stimulated by the addition of aniline.

Catechol dioxygenase. Cell-free extracts were prepared from aniline-grown cultures, and these were tested for the presence of catechol-1,2-oxygenase and catechol-2,3-oxygenase activity. No catechol-1,2-oxygenase activity was detected, but catechol-2,3-oxygenase activity was 98 μmoles/min/mg protein.

The levels of catechol-2,3-dioxygenase were determined in cultures of strain K1 grown on various substrates (Table 3). No dioxygenase activity was found in cultures grown in the absence of aniline. However, cultures grown on a mixture of aniline and an organic acid did contain catechol-2,3-oxygenase.

Aniline utilization in mixed-substrate cultures. Catechol dioxygenase was induced by aniline, even in cultures where lactate was present. To determine if aniline and lactate were used simultaneously, K1 was grown on 16 mM lactate plus a low concentration of aniline (1 mM). [^{14}C]-aniline was added as a radiotracer to monitor aniline metabolism.

Diauxic growth was not observed in the mixed substrate culture (Fig. 3). Furthermore, the results suggest that lactate and aniline were used simultaneously. On 1 mM aniline alone, strain K1 has consumed all of the substrate at a turbidity of 0.125. On 16 mM lactate alone, stationary phase of a batch culture occurs at a turbidity of 3.0. If aniline were used preferentially to lactate in the mixed-substrate culture, it would have been

depleted at 8 hours, when the OD equalled 0.125. However, only 25% of the aniline had been consumed at this time. If lactate were used preferentially to aniline, then no aniline metabolism would have been observed during the time of this experiment, yet all aniline was metabolized by 12 hours.

Induction of aniline metabolism. Strain K1 was shown to be incapable of growth on aniline analogs. The respirometry experiments indicate that analogs are poor substrates for at least some of the enzymes that catabolize aniline. However, another possible factor for the absence of growth is that these substrates do not induce the catabolic enzymes necessary for growth on anilines. To test this, 1 mM concentrations of putative inducers were added to log phase cultures of K1 growing on lactate. After 3 h, the cells were harvested, washed, and tested for their ability to metabolize [^{14}C]-aniline (Table 4). 4-Chloroaniline was as good an inducer as aniline itself, and some induction was noted with 3-chloroaniline. The addition of 2-methylaniline or 2,6-diethylaniline did not increase aniline metabolism above that found in the absence of an inducer.

DISCUSSION

Strain K1 was isolated as a strain that could grow relatively rapidly on high concentrations of anilines in the absence of growth factors. The generation time on 8 mM aniline was 2.2 h, comparable to that found for Pseudomonas putida UCC2 (McClure and Venables, 1986). Pseudomonas multivorans An1 (Helm and Reber, 1979) had a doubling time of 2 h in 1 mM aniline, but at 8 mM its generation time increased to 7 h. The generation times of several other aniline-degrading bacteria have been reported to be in excess of 6 h (Zeyer and Kearney, 1982; Surovtseva and Vol'nova, 1980).

Strain K1 was able to metabolize aniline at low concentrations. Measurable uptake occurred at the lowest concentration tested -- 180 nM. The half-saturation constant for uptake derived from the Michaelis-Menten equation was 3.8 micromolar, a value in the lowest 20% of kinetic constants cited by Button (1985) for transport of organic carbon molecules. During the 20 min incubation period with [^{14}C]-aniline, relatively little of the carbon was assimilated into cell material. This probably occurred because the intracellular pool of biosynthetic intermediates had not reached isotopic equilibrium with the external pool of aniline during the incubation (King and Berman, 1984).

The aerobic metabolism of diverse aromatic compounds proceeds by the formation of a catechol, which then serves as a substrate for a ring-fission dioxygenase (Dagley, 1986). In several aniline-degrading bacteria, aniline was shown to be metabolized via oxidative deamination (Bachofer et al., 1975; Walker and Harris, 1969; McClure and Venables, 1986). These organisms are simultaneously adapted (Stanier, 1947) to the oxidation of catechol when they are grown on aniline (Zeyer and Kearney, 1982; Surovtseva and Vol'nova, 1980; Surovtseva et al., 1985; Wyndham, 1986). Furthermore, extracts of aniline-grown cells contain either catechol-1,2-oxygenase or catechol-2,3-oxygenase activity (Kaminski et al., 1983; McClure and Venables, 1986; Anson and Mackinnon, 1984; Zeyer et al., 1985; Wyndham, 1986). Strain K1 exhibited similar properties. Aniline-grown cells oxidized catechol as well as aniline, and catechol-2,3-oxygenase activity was measured in cell extracts. This enzyme is characteristic of the meta-pathway of aromatic metabolism.

Strain K1 exhibited a narrow range of aromatic substrate utilization. No aromatic substrate other than aniline was used for growth, with the exception of aromatic amino acids. Utilization of aromatic amino acids is found in a

wide variety of microorganisms. Several aminoaromatics stimulated respiration of resting cells of K1, but the rates were much less than was found upon addition of aniline. The inability to use halogenated anilines is not surprising, given the use of the meta pathway by strain K1. Metabolism of haloaromatics by the meta pathway produces dead-end metabolites (Knackmuss, 1981). Thus, all strains that have been found to degrade chloroanilines use the ortho pathway (Zeyer and Kearney, 1982a; Zeyer and Kearney, 1982b; Surovtseva et al., 1985; You and Bartha, 1982).

Other aniline-degrading microbes that have been isolated are similar to K1 in that they do not grow on a wide range of aromatic substrates (Walker and Harris, 1969; Surovtseva and Vol'nova, 1980). Wyndham (1986) isolated strains of Acinetobacter calcoaceticus that grew on phenol in addition to aniline. The selection of variants of Pseudomonas putida mt-2 which grew on aniline or toluidine resulted in the loss of the upper TOL pathway in the organisms, and therefore these variants could no longer grow on toluene, xylene, or toluate (McClure and Venables, 1986).

The synthesis of the enzymes for catabolism of an aromatic substrate generally requires the presence of an inducer, which is either the aromatic substrate itself (Worsey et al., 1978) or an intermediate in the degradative pathway (Ornston, 1971). In most aniline-degrading microbes, aniline degradation has been reported to be inducible (Wyndham, 1986; Anson and MacKinnon, 1984; Walker and Harris, 1969; McClure and Venables, 1986). However, in Rhodococcus An117, catechol dioxygenase was constitutive; the capacity to convert aniline to catechol required induction by aniline (Kaminski et al., 1983).

In strain K1, the degradation of aniline was an inducible system, as shown by the inability of cells grown on lactate or acetate to respire when

given aniline (Table 1) and by the absence of catechol dioxygenase in those cells (Table 3). Two chlorinated aniline derivatives could also induce aniline metabolism; this result is similar to that of Kaminski et al. (1983), who found that aniline metabolism in Rhodococcus An117 was induced by chloroanilines, even though the organism could not grow on them. In Moraxella strain G, aniline oxidation was induced by a wide variety of halogenated anilines, some of which supported growth of the organism (Zeyer et al., 1985).

In several other aniline-degrading microbes, catabolism of aniline is repressed by the presence of other organic substrates (Zeyer et al., 1985; Helm and Reber, 1979). Similar phenomena have been observed with other aromatic substrates (Heiman and Cooper, 1987; Schmidt and Alexander, 1985). However, strain K1 was able to synthesize catechol dioxygenase and to metabolize aniline in the presence of high concentrations of lactate. In general, diauxic growth is observed in the presence of two substrates when there is a saturating concentration of a "good" substrate; that is, one which supports a faster growth rate than the second substrate (Harder and Dijkhuizen, 1982). The results obtained with strain K1 are consistent with this idea. Aniline supports as fast a growth rate as that found with other organic substrates and therefore is not a "poor" substrate for growth.

The simultaneous utilization of aniline and other organic substrates by strain K1 has significance in the biodegradation of aniline in natural environments. If other organic substrates are present in high concentration, they could repress aniline degradation in strains susceptible to catabolite repression. Strain K1 may continue to degrade aniline in such environments. Conversely, in groundwater systems, the concentration of xenobiotic may be too low to support growth of a microbial population which can transform it at a high rate. However, if an organism such as strain K1 is present which can

simultaneously utilize the xenobiotic and a second natural substrate, higher population levels could be supported by introducing a secondary substrate. This increased population could then transform the xenobiotic at an accelerated rate.

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Table 1. Aromatic substrate utilization by strain K1. The substrates were tested as sole carbon and energy sources for growth or for their ability to stimulate oxygen uptake by resting cells of K1 that had been grown on aniline.

Substrate	Growth	Respiration rate[a]
Aniline	+	47
Catechol	-	155
3,4-Dihydroxybenzylamine	-	16
Toluidine	-	15
2-Aminophenol	-	0
3-Aminophenol	-	12
4-Aminophenol	-	3
4-Aminobenzoate	-	2
3-Chloroaniline	-	
4-Chloroaniline	-	0
3,4-Dichloroaniline	-	0
2,6-Diethylaniline	-	0
Phenol	-	0
Benzoate	-	0
Toluene	-	0
Pyridine	-	0
Anthranilate	-	0
Tryptophan	+	0
4-Hydroxybenzoate	-	0

[a] nmol O_2/min/10^9 cells grown on 8 mM aniline.

Table 2. Substrate-dependent oxygen uptake by resting cell suspensions of strain K1 grown on different organic substrates.

Substrate	Respiration rate (nmol O_2/min/10^9 cells) for cells grown on		
	Aniline	Lactate	Acetate
Aniline	44	0	0
Acetate	9	6	50
Lactate	9	24	17

Table 3. Catechol dioxygenase activity in strain K1 grown on various substrates.

Growth substrate(s)	Catechol-2,3-oxygenase activity (μmmol/min/mg protein)
Aniline	110
Acetate	0
Lactate	0
Aniline + acetate	93
Aniline + lactate	66

Table 4. Rate of aniline oxidation by strain K1 after 3 hours exposure to 1 mM concentrations of putative inducers.

Inducer	[^{14}C]-Aniline metabolized[a] (pmol/ml/h)
None	860
Aniline	4100
2-Methylaniline	480
3-Chloroaniline	2800
4-Chloroaniline	4300
2,6-Diethylaniline	1060

[a]Rate for 7 x 10^6 cells/ml at an aniline concentration of 10 μM.

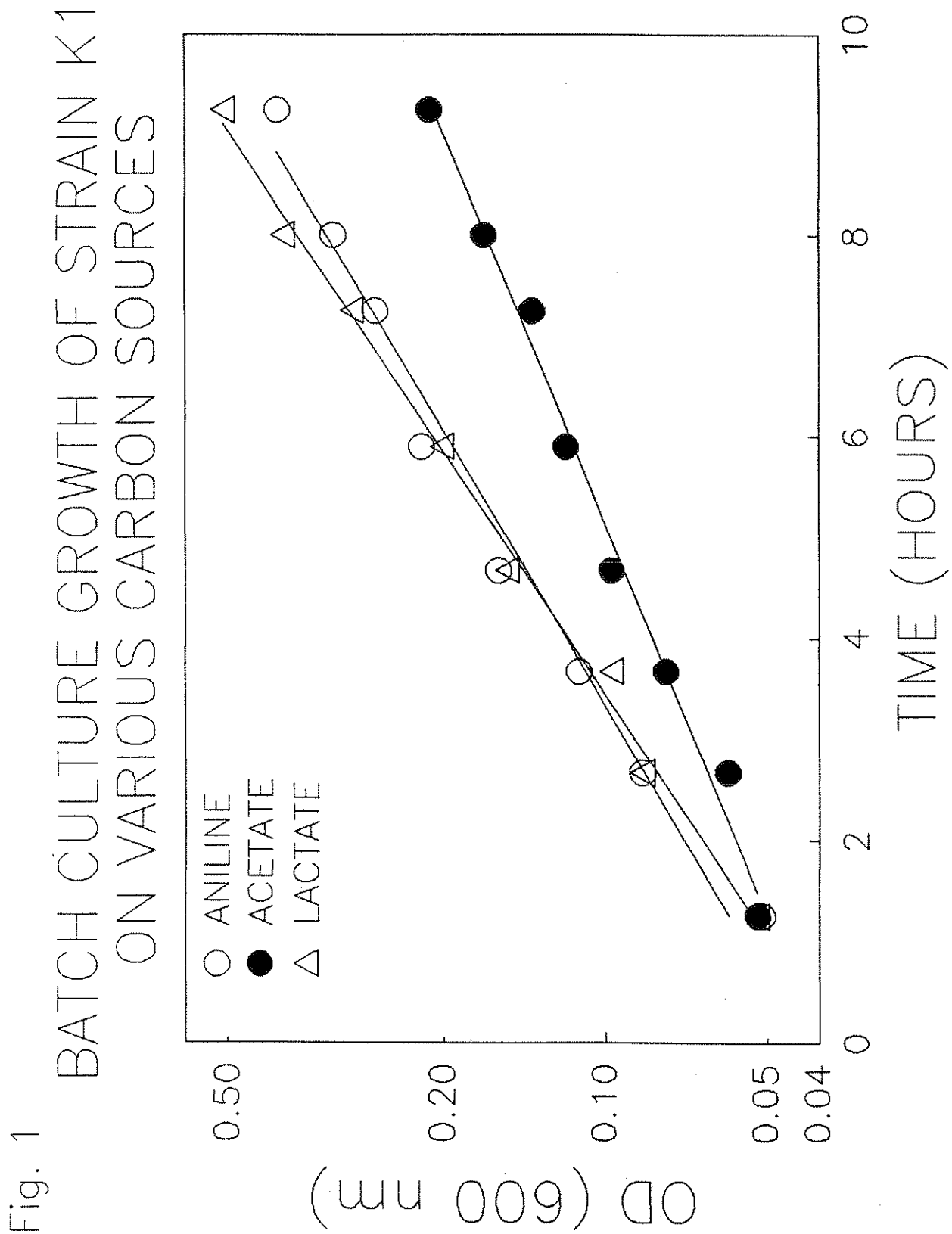
FIGURE LEGENDS

Fig. 1. Growth of strain K1 in batch cultures of XBM salts containing (○) 8 mM aniline, (△) 16 mM lactate, or (●) 25 mM acetate.

Fig. 2. Kinetics of aniline uptake by strain K1 grown on 8 mM aniline. Metabolism of [^{14}C]-aniline into particulate material and CO_2 was determined at a series of aniline concentrations, in cell suspensions at a density of 7 x 10^6 cells/ml.

Fig. 3. Growth of strain K1 in XBM containing 16 mM lactate and 1 mM aniline. In addition to turbidity (○), the concentration of aniline (△) in the culture was monitored by measuring the formation of ^{14}C-particulate material and $^{14}CO_2$ produced from 0.02 microcuries [^{14}C]-aniline added per ml culture as a radiotracer.

Fig. 1


BATCH CULTURE GROWTH OF STRAIN K1
ON VARIOUS CARBON SOURCES
ANILINE
ACETATE
LACTATE
OD (600 nm)
0.50
0.20
0.10
0.05
0.04
0
2
4
6
8
10
TIME (HOURS)

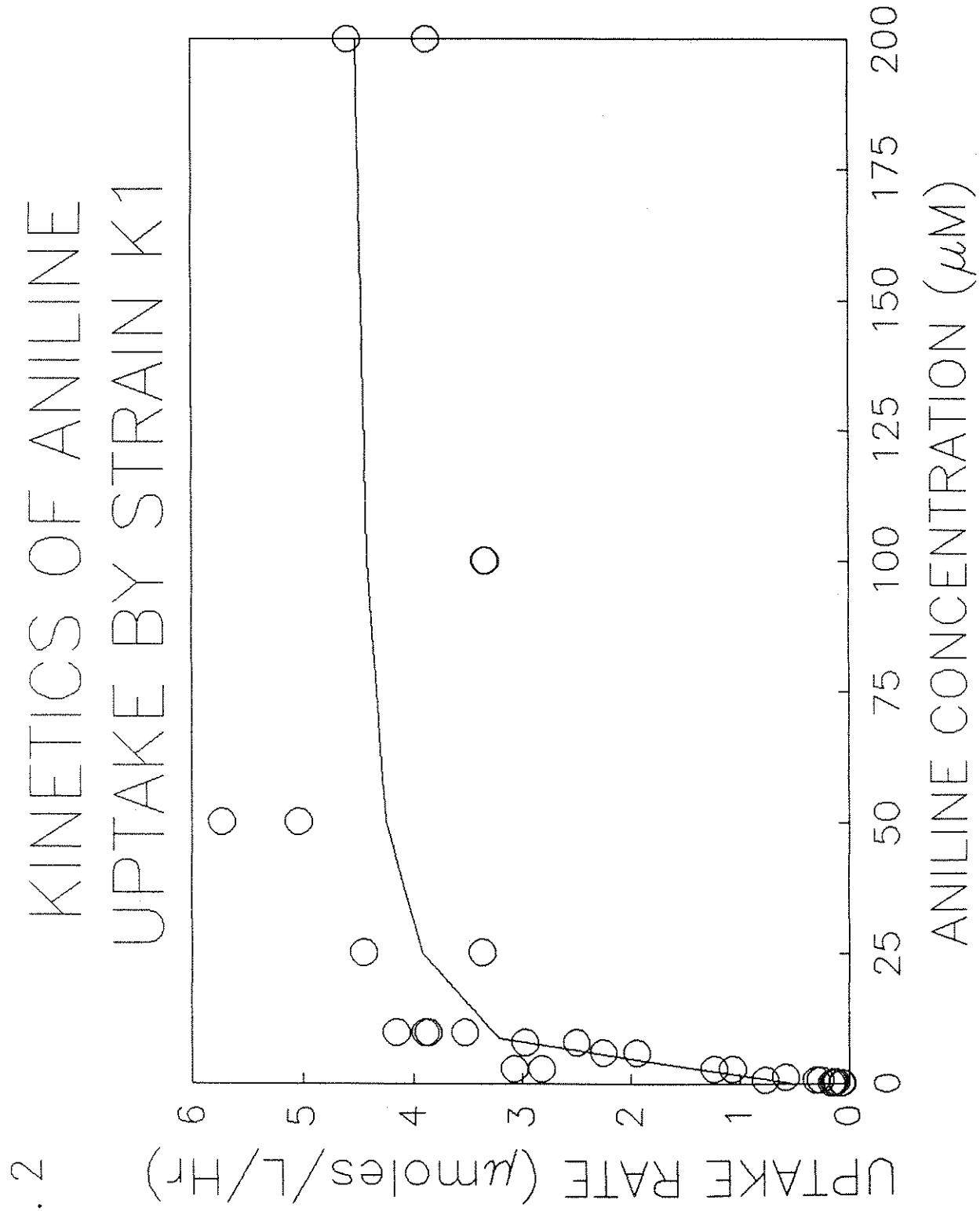

KINETICS OF ANILINE
UPTAKE BY STRAIN K1
UPTAKE RATE (μmoles/L/Hr)
0
1
2
3
4
5
6
0
25
50
75
100
125
150
175
200
ANILINE CONCENTRATION (μM)

Fig. 2

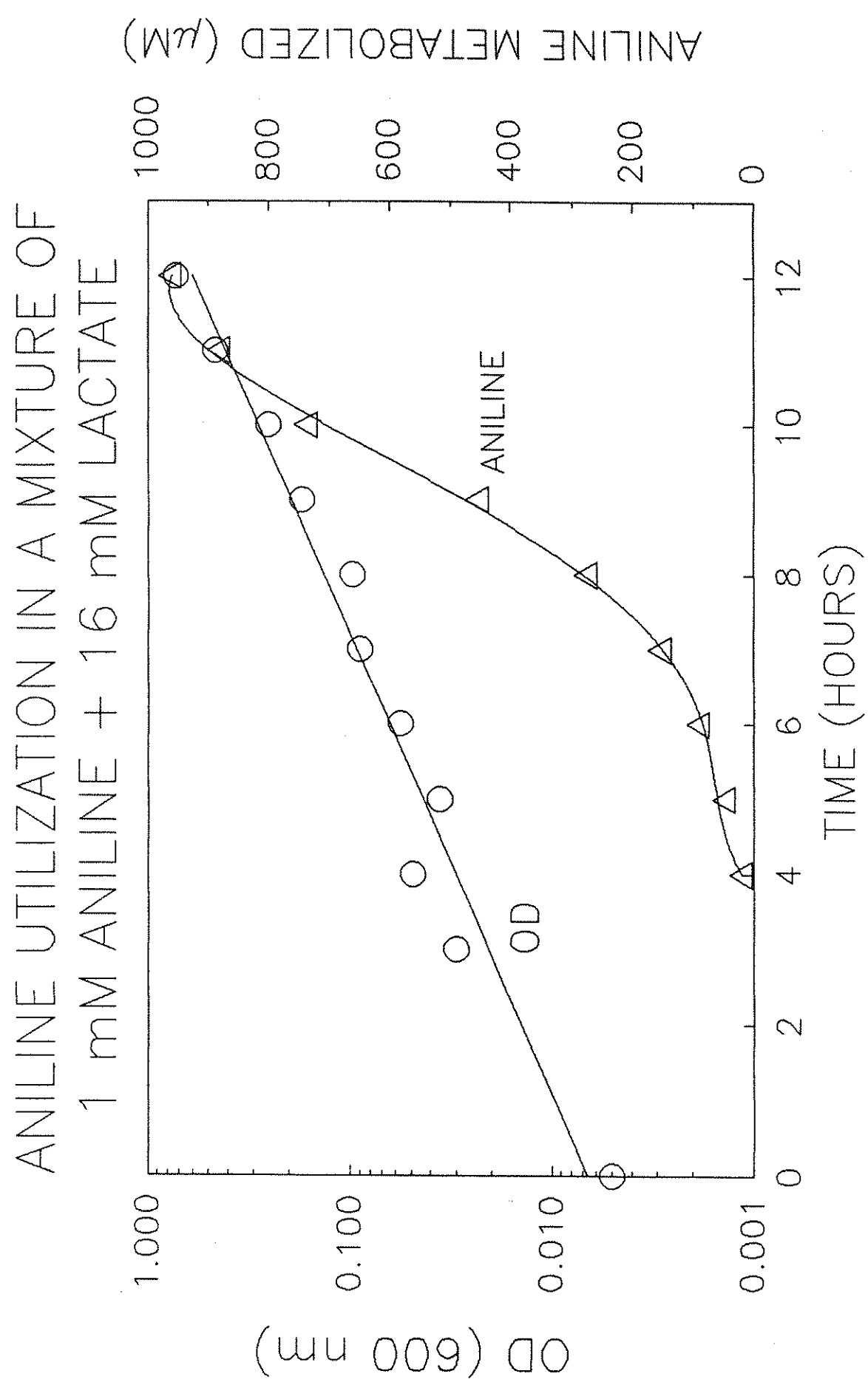

ANILINE UTILIZATION IN A MIXTURE OF
1 mM ANILINE + 16 mM LACTATE
OD (600 nm)
ANILINE METABOLIZED (μM)
TIME (HOURS)
ANILINE
OD
1.000
0.100
0.010
0.001
0
200
400
600
800
1000
2
4
6
8
10
12

Fig. 3